Class18

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Q1. With the help of the R "addin" package datapasta assign the CDC pertussis case number data to a data frame called cdc and use ggplot to make a plot of cases numbers over time.

```
library(datapasta)
```

```
Warning: package 'datapasta' was built under R version 4.4.3
```

```
library(janitor)
```

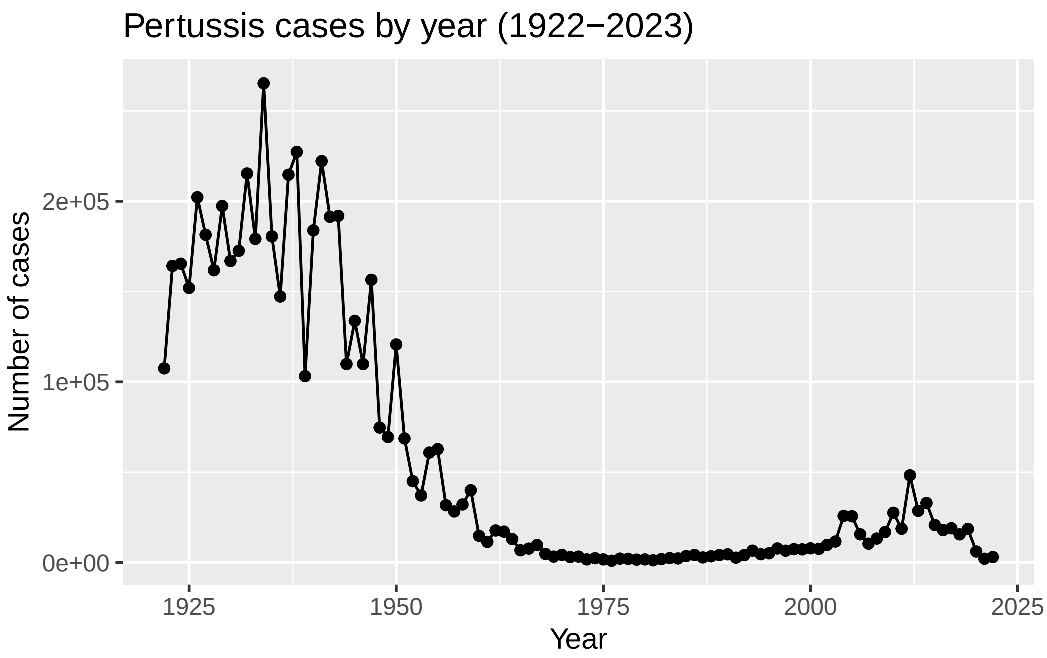
```

Attaching package: 'janitor'
```

```
The following objects are masked from 'package:stats':

    chisq.test, fisher.test
```

```
cdc <- data.frame(
                                 Year = c(1922L,1923L,1924L,1925L,
                                          1926L,1927L,1928L,1929L,1930L,1931L,
                                          1932L,1933L,1934L,1935L,1936L,
                                          1937L,1938L,1939L,1940L,1941L,1942L,
                                          1943L,1944L,1945L,1946L,1947L,
                                          1948L,1949L,1950L,1951L,1952L,
                                          1953L,1954L,1955L,1956L,1957L,1958L,
                                          1959L,1960L,1961L,1962L,1963L,
                                          1964L,1965L,1966L,1967L,1968L,1969L,
                                          1970L,1971L,1972L,1973L,1974L,
                                          1975L,1976L,1977L,1978L,1979L,1980L,
                                          1981L,1982L,1983L,1984L,1985L,
                                          1986L,1987L,1988L,1989L,1990L,
                                          1991L,1992L,1993L,1994L,1995L,1996L,
                                          1997L,1998L,1999L,2000L,2001L,
                                          2002L,2003L,2004L,2005L,2006L,2007L,
                                          2008L,2009L,2010L,2011L,2012L,
                                          2013L,2014L,2015L,2016L,2017L,2018L,
                                          2019L,2020L,2021L,2022L),
         No..Reported.Pertussis.Cases = c(107473,164191,165418,152003,
                                          202210,181411,161799,197371,
                                          166914,172559,215343,179135,265269,
                                          180518,147237,214652,227319,103188,
                                          183866,222202,191383,191890,109873,
                                          133792,109860,156517,74715,69479,
                                          120718,68687,45030,37129,60886,
                                          62786,31732,28295,32148,40005,
                                          14809,11468,17749,17135,13005,6799,
                                          7717,9718,4810,3285,4249,3036,
                                          3287,1759,2402,1738,1010,2177,2063,
                                          1623,1730,1248,1895,2463,2276,
                                          3589,4195,2823,3450,4157,4570,
                                          2719,4083,6586,4617,5137,7796,6564,
                                          7405,7298,7867,7580,9771,11647,
                                          25827,25616,15632,10454,13278,
                                          16858,27550,18719,48277,28639,32971,
                                          20762,17972,18975,15609,18617,
                                          6124,2116,3044)
       )

#clean up the column names of the dataframe
cdc <- clean_names(cdc)
head(cdc)
```

```
  year no_reported_pertussis_cases
1 1922                      107473
2 1923                      164191
3 1924                      165418
4 1925                      152003
5 1926                      202210
6 1927                      181411
```

```
library(ggplot2)
ggplot(cdc) +
  aes(x=year, y = no_reported_pertussis_cases) +
  geom_line() +
  geom_point() +
  labs(x = "Year", y = "Number of cases", title = "Pertussis cases by year (1922-2023)")
```

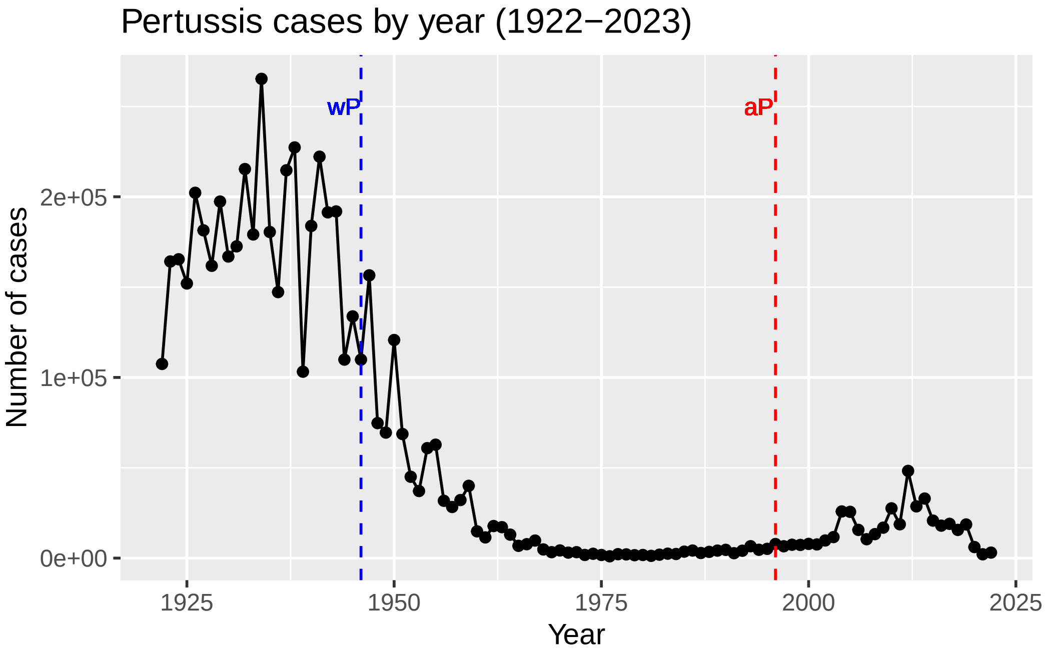


Q2. Using the ggplot geom_vline() function add lines to your previous plot for the 1946 introduction of the wP vaccine and the 1996 switch to aP vaccine (see example in the hint below). What do you notice?

```
ggplot(cdc) +
  aes(x=year, y = no_reported_pertussis_cases) +
  geom_line() +
  geom_point() +
  labs(x = "Year", y = "Number of cases", title = "Pertussis cases by year (1922-2023)") +
  geom_vline(xintercept = 1946, col = "blue", linetype = "dashed") +
  geom_vline(xintercept = 1996, col = "red", linetype = "dashed") +
  geom_text(aes(x = 1944, y = 250000, label = "wP"), color = "blue", size = 3) +
  geom_text(aes(x = 1994, y = 250000, label = "aP"), color = "red", size = 3)
```

```
Warning in geom_text(aes(x = 1944, y = 250000, label = "wP"), color = "blue", : All aesthetic
i Please consider using `annotate()` or provide this layer with data containing
  a single row.
```

```
Warning in geom_text(aes(x = 1994, y = 250000, label = "aP"), color = "red", : All aesthetic
i Please consider using `annotate()` or provide this layer with data containing
  a single row.
```



After the introduction of the wP vaccine in 1946, the number of pertussis cases quickly drops and then plateaus. After the switch to the aP vaccine in 1996, there is a slight increase in cases and in general, more variability with the number of cases per year.

Q3. Describe what happened after the introduction of the aP vaccine? Do you have a possible explanation for the observed trend?

fter the switch to the aP vaccine in 1996, there is an increase in cases per year and in general, more variability with the number of cases per year. Some possible reasons for this increase could be that the aP vaccine is not quite as effective as the wP vaccine. There could also be better detection of pertussis cases or increaesed reporting of pertussis cases. Finally, the anti-vax movement starting picking up steam right around when the pertussis cases start to rise, so it could be from people not vaccinating their children.

```
library(jsonlite)
```

```
Warning: package 'jsonlite' was built under R version 4.4.3
```

```
subject <- read_json("https://www.cmi-pb.org/api/subject", simplifyVector = TRUE)
head(subject,3)
```

```
  subject_id infancy_vac biological_sex               ethnicity  race
1          1          wP         Female Not Hispanic or Latino White
2          2          wP         Female Not Hispanic or Latino White
3          3          wP         Female                 Unknown White
  year_of_birth date_of_boost      dataset
1    1986-01-01    2016-09-12 2020_dataset
2    1968-01-01    2019-01-28 2020_dataset
3    1983-01-01    2016-10-10 2020_dataset
```

Q3. How many subjects are in the dataset?

```
nrow(subject)
```

```
[1] 172
```

172 subjects are in the dataset

Q4. How many aP and wP infancy vaccinated subjects are in the dataset?

```
table(subject$infancy_vac)
```

```

aP wP 
87 85 
```

There are 87 aP infancy vaccinated subjects and 85 wP infancy vaccinated subjects

Q5. How many Male and Female subjects/patients are in the dataset?

```
table(subject$biological_sex)
```

```

Female   Male 
   112     60 
```

There are 112 female patients and 60 male patients

Q6. What is the breakdown of race and biological sex (e.g. number of Asian females, White males etc…)?

```
table(subject$race, subject$biological_sex)
```

```
                                            Female Male
  American Indian/Alaska Native                  0    1
  Asian                                         32   12
  Black or African American                      2    3
  More Than One Race                            15    4
  Native Hawaiian or Other Pacific Islander      1    1
  Unknown or Not Reported                       14    7
  White                                         48   32
```

```
library(lubridate)
```

```

Attaching package: 'lubridate'

The following objects are masked from 'package:base':

    date, intersect, setdiff, union
```

```
today()
```

```
[1] "2025-03-08"
```

```
today() - ymd("2000-01-01")
```

```
Time difference of 9198 days
```

```
time_length( today() - ymd("2000-01-01"),  "years")
```

```
[1] 25.18275
```

Q7. Using this approach determine (i) the average age of wP individuals, (ii) the average age of aP individuals; and (iii) are they significantly different?

```r
library(dplyr)
```

```
Attaching package: 'dplyr'


The following objects are masked from 'package:stats':

    filter, lag


The following objects are masked from 'package:base':

    intersect, setdiff, setequal, union
```

```r
subject$age <- time_length(today() -
                             ymd(subject$year_of_birth), "years")
wp <- subject %>%
  filter(infancy_vac == "wP")
avg_age_wp <- mean(wp$age)
cat("average age for wP: ", avg_age_wp)
```

```
average age for wP:  35.8288
```

```r
ap <- subject %>%
  filter(infancy_vac == "aP")
avg_age_ap <- mean(ap$age)
cat("average age for aP: ", avg_age_ap)
```

```
average age for aP:  27.0781
```

```r
t.test(wp$age, ap$age)
```

```

    Welch Two Sample t-test

data:  wp$age and ap$age
t = 12.918, df = 104.03, p-value < 2.2e-16
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
  7.407351 10.094058
sample estimates:
mean of x mean of y
  35.8288   27.0781
```

The average age of wP individuals is 36 years and the average age of aP individuals is 27 years and the difference is significant since the p value is less than 0.05

Q8. Determine the age of all individuals at time of boost?

```
subject$boost_age <- time_length(ymd(subject$date_of_boost) - ymd(subject$year_of_birth), "ye
subject$boost_age[1:5]
```

```
[1] 30.69678 51.07461 33.77413 28.65982 25.65914
```

The code gets cut off in the rendered version but it is:

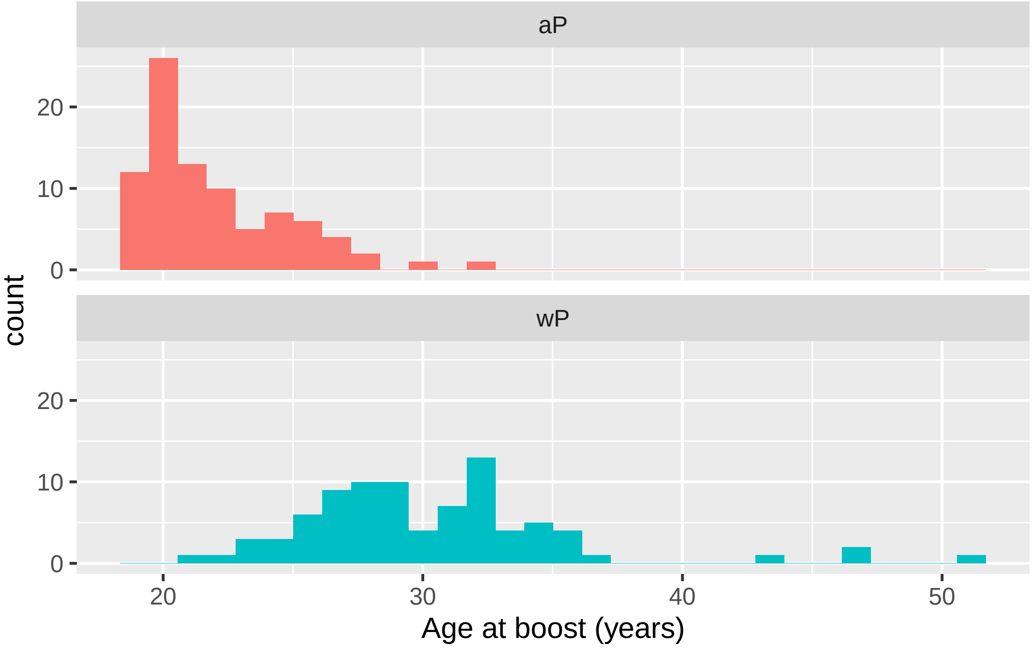
subject$boost_age < -time_length(ymd(subject$date_of_boost) -
ymd(subject$year_of_birth), “years”)

subject$boost_age[1:5]

Q9. With the help of a faceted boxplot or histogram (see below), do you think these two groups are significantly different?

```
ggplot(subject) +
  aes(x=boost_age, fill = infancy_vac) +
  geom_histogram(show.legend = FALSE) +
  facet_wrap(~infancy_vac, nrow = 2) +
  labs(x="Age at boost (years)")
```

```
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```



These groups seem very different since the distribution of ages at time of boost for aP vaccine is shifted much younger than the distribution for boost ages for the wP vaccine.

```
# Complete the API URLs...
specimen <- read_json("https://www.cmi-pb.org/api/specimen", simplifyVector = TRUE)
titer <- read_json("https://www.cmi-pb.org/api/plasma_ab_titer", simplifyVector = TRUE)
```

Q9. Complete the code to join specimen and subject tables to make a new merged data frame containing all specimen records along with their associated subject details:

```
meta <- inner_join(specimen, subject)
```

```
Joining with `by = join_by(subject_id)`
```

```
dim(meta)
```

```
[1] 1503   15
```

```
head(meta)
```

```
  specimen_id subject_id actual_day_relative_to_boost
1           1          1                           -3
2           2          1                            1
3           3          1                            3
4           4          1                            7
5           5          1                           11
6           6          1                           32
  planned_day_relative_to_boost specimen_type visit infancy_vac biological_sex
1                             0         Blood     1          wP         Female
2                             1         Blood     2          wP         Female
3                             3         Blood     3          wP         Female
4                             7         Blood     4          wP         Female
5                            14         Blood     5          wP         Female
6                            30         Blood     6          wP         Female
               ethnicity  race year_of_birth date_of_boost      dataset
1 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
2 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
3 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
4 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
5 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
6 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
       age boost_age
1 39.18138  30.69678
2 39.18138  30.69678
3 39.18138  30.69678
4 39.18138  30.69678
5 39.18138  30.69678
6 39.18138  30.69678
```

Q10. Now using the same procedure join meta with titer data so we can further analyze this data in terms of time of visit aP/wP, male/female etc.

```r
abdata <- inner_join(titer, meta)
```

```
Joining with `by = join_by(specimen_id)`
```

```r
dim(abdata)
```

```
[1] 52576    22
```

Q11. How many specimens (i.e. entries in abdata) do we have for each isotype?

```
table(abdata$isotype)
```

```
  IgE   IgG  IgG1  IgG2  IgG3  IgG4 
 6698  5389 10117 10124 10124 10124 
```

Q12. What are the different $dataset values in abdata and what do you notice about the number of rows for the most "recent" dataset?

```
table(abdata$dataset)
```

```
2020_dataset 2021_dataset 2022_dataset 2023_dataset 
       31520         8085         7301         5670 
```

The different dataset values are which year the data is from. The most recent dataset value is the lowest so it has the fewest number of rows.

```
igg <- abdata %>% filter(isotype == "IgG")
head(igg)
```

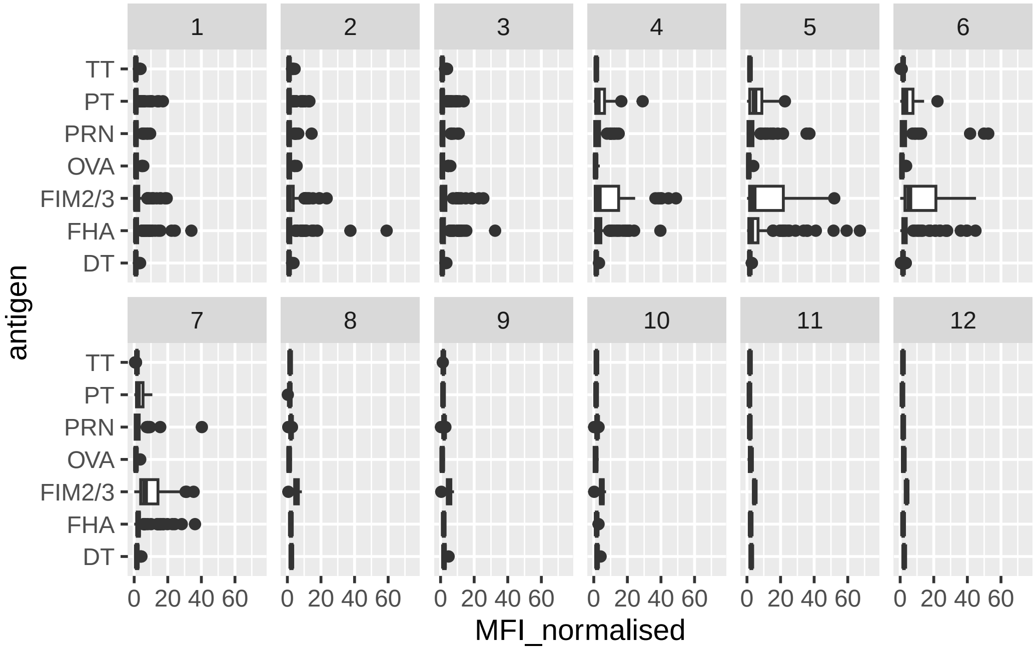
```
  specimen_id isotype is_antigen_specific antigen        MFI MFI_normalised
1           1     IgG                TRUE      PT   68.56614       3.736992
2           1     IgG                TRUE     PRN  332.12718       2.602350
3           1     IgG                TRUE     FHA 1887.12263      34.050956
4          19     IgG                TRUE      PT   20.11607       1.096366
5          19     IgG                TRUE     PRN  976.67419       7.652635
6          19     IgG                TRUE     FHA   60.76626       1.096457
   unit lower_limit_of_detection subject_id actual_day_relative_to_boost
1 IU/ML                 0.530000          1                           -3
2 IU/ML                 6.205949          1                           -3
3 IU/ML                 4.679535          1                           -3
4 IU/ML                 0.530000          3                           -3
5 IU/ML                 6.205949          3                           -3
6 IU/ML                 4.679535          3                           -3
  planned_day_relative_to_boost specimen_type visit infancy_vac biological_sex
1                             0         Blood     1          wP         Female
2                             0         Blood     1          wP         Female
3                             0         Blood     1          wP         Female
4                             0         Blood     1          wP         Female
```

```
5                             0         Blood     1          wP        Female
6                             0         Blood     1          wP        Female
               ethnicity  race year_of_birth date_of_boost      dataset
1 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
2 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
3 Not Hispanic or Latino White    1986-01-01    2016-09-12 2020_dataset
4                Unknown White    1983-01-01    2016-10-10 2020_dataset
5                Unknown White    1983-01-01    2016-10-10 2020_dataset
6                Unknown White    1983-01-01    2016-10-10 2020_dataset
       age boost_age
1 39.18138  30.69678
2 39.18138  30.69678
3 39.18138  30.69678
4 42.18207  33.77413
5 42.18207  33.77413
6 42.18207  33.77413
```

Q13. Complete the following code to make a summary boxplot of Ab titer levels (MFI) for all antigens:

```r
ggplot(igg) +
  aes(MFI_normalised, antigen) +
  geom_boxplot() +
    xlim(0,75) +
  facet_wrap(vars(visit), nrow=2)
```

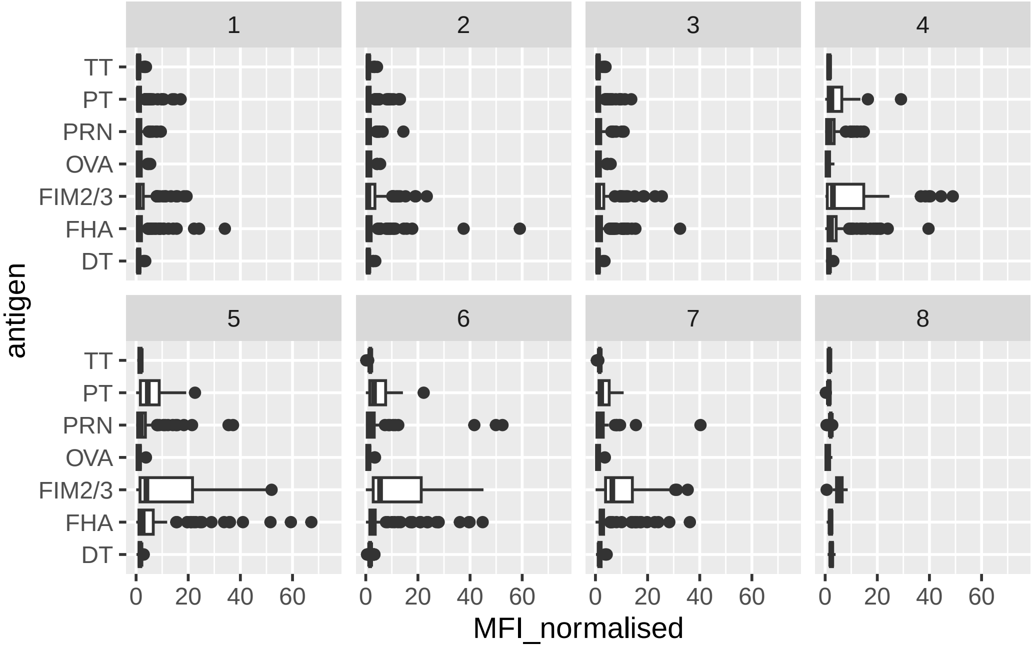
```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```



doing the same, but only looking at 8 visits

```
igg_8 <- igg %>% filter(visit < 9)
ggplot(igg_8) +
  aes(MFI_normalised, antigen) +
  geom_boxplot() +
    xlim(0,75) +
  facet_wrap(vars(visit), nrow=2)
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```

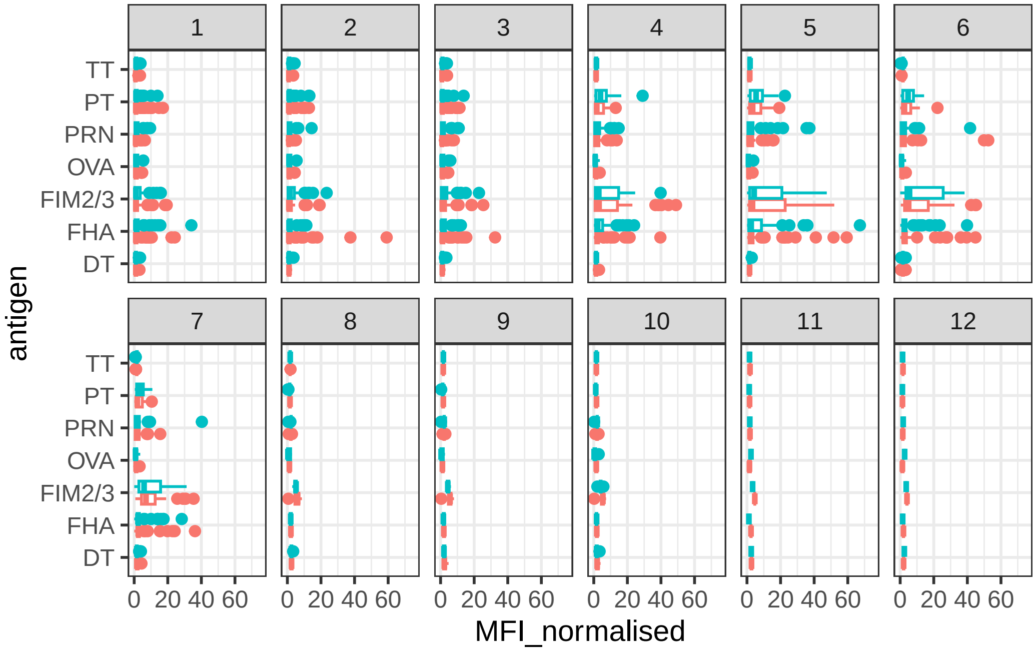


Q14. What antigens show differences in the level of IgG antibody titers recognizing them over time? Why these and not others?

PT, FIM2/3, and FHA increase up until the 7th visit and then decrease after that. Most of the others stay around the same titer each visit. PRN also has a slight increase. The antigens that increased over time are related to pertussis (PT = pertussis toxin, FIM2/3 =fimbrial protein 2/3 which is from pertussis, FHA = Filamentous hemagglutinin from pertussis, PRN = pertactin autotransporter from pertussis). The other antigens are related to other infections (TT = tetanus toxin, DT = diptheria toxin) or unrelated to infection (OVA = ovalbumin)

```r
ggplot(igg) +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  xlim(0,75) +
  theme_bw()
```

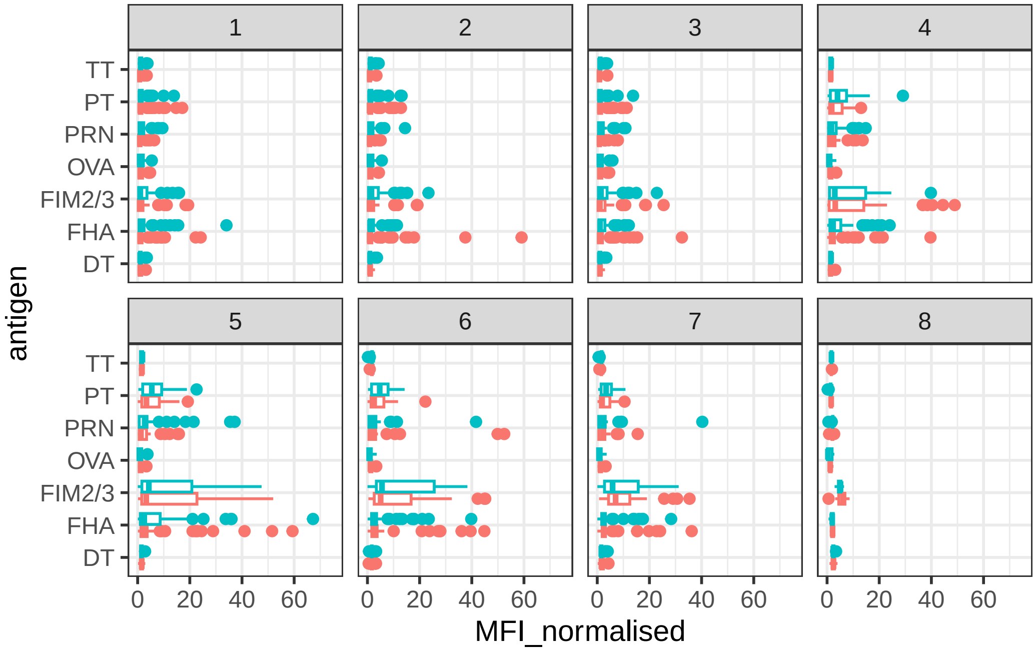
```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```



doing the same, but only looking at 8 visits

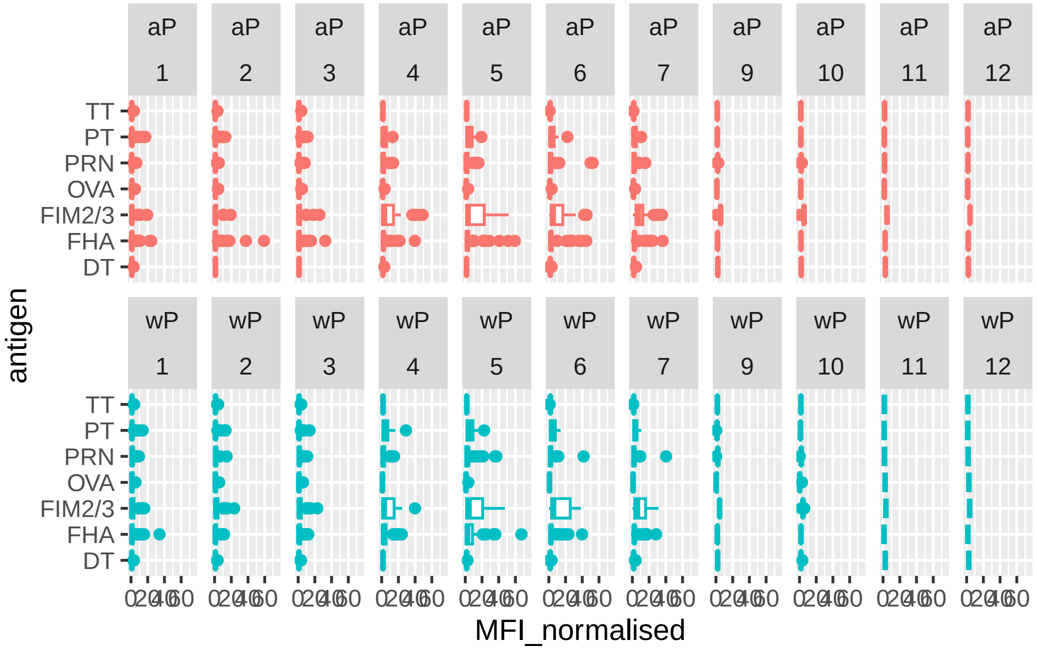
```
ggplot(igg_8) +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit), nrow=2) +
  xlim(0,75) +
  theme_bw()
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```



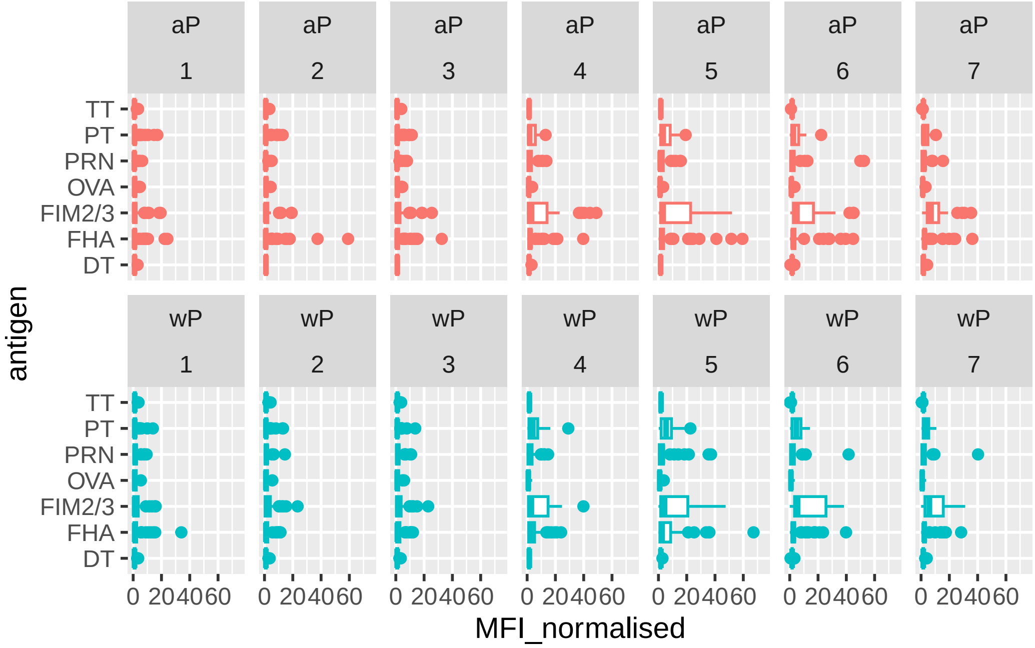
```
igg %>% filter(visit != 8) %>%
ggplot() +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  xlim(0,75) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```



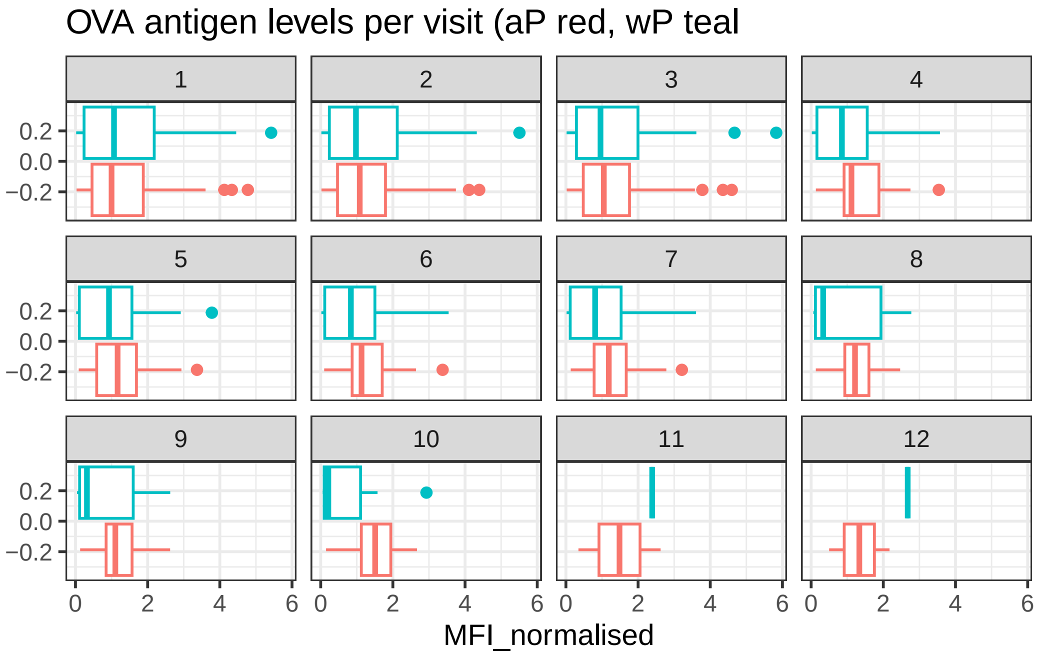
```
igg_8 %>% filter(visit != 8) %>%
ggplot() +
  aes(MFI_normalised, antigen, col=infancy_vac ) +
  geom_boxplot(show.legend = FALSE) +
  xlim(0,75) +
  facet_wrap(vars(infancy_vac, visit), nrow=2)
```

```
Warning: Removed 5 rows containing non-finite outside the scale range
(`stat_boxplot()`).
```

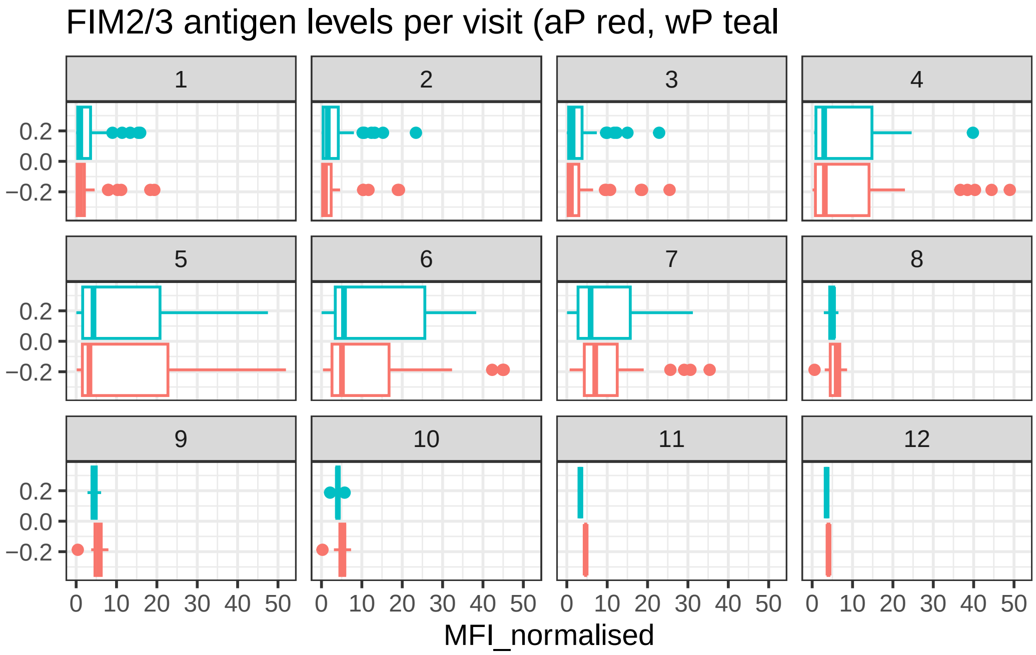


Q15. Filter to pull out only two specific antigens for analysis and create a boxplot for each. You can chose any you like. Below I picked a "control" antigen ("OVA", that is not in our vaccines) and a clear antigen of interest ("PT", Pertussis Toxin, one of the key virulence factors produced by the bacterium B. pertussis).

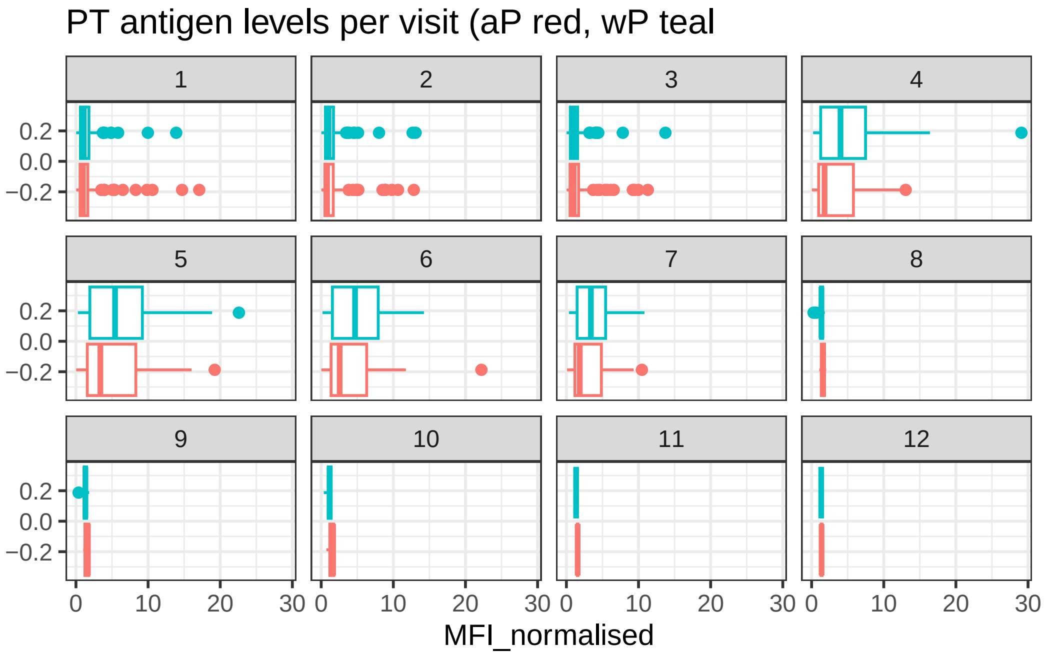
```
filter(igg, antigen=="OVA") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  labs(title = "OVA antigen levels per visit (aP red, wP teal")
```



```
filter(igg, antigen=="FIM2/3") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  labs(title = "FIM2/3 antigen levels per visit (aP red, wP teal")
```

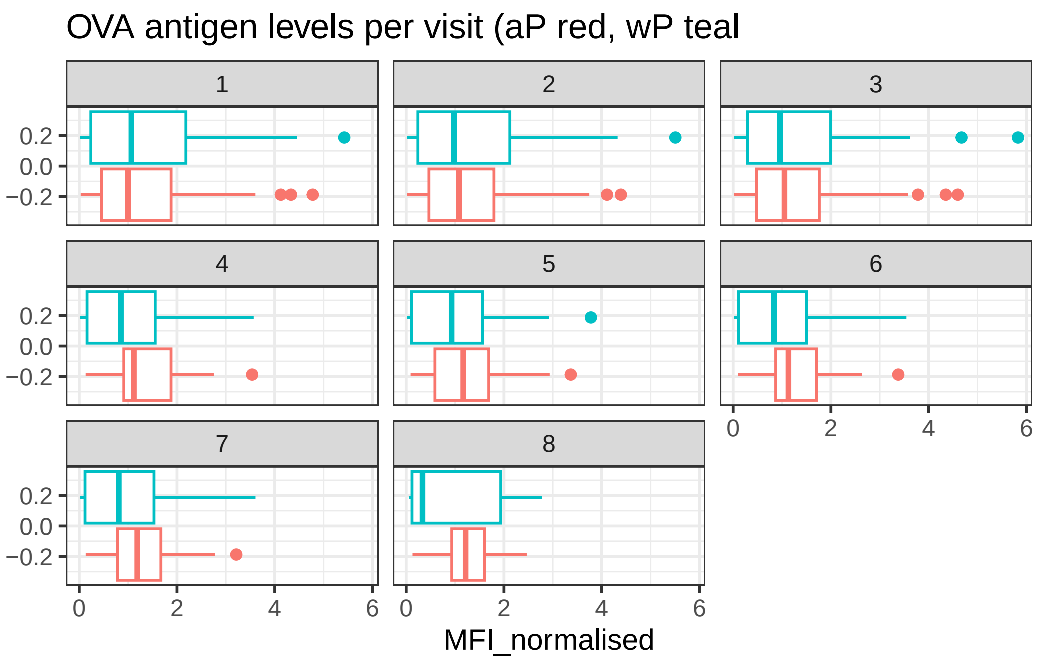


```r
filter(igg, antigen=="PT") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  labs(title = "PT antigen levels per visit (aP red, wP teal")
```

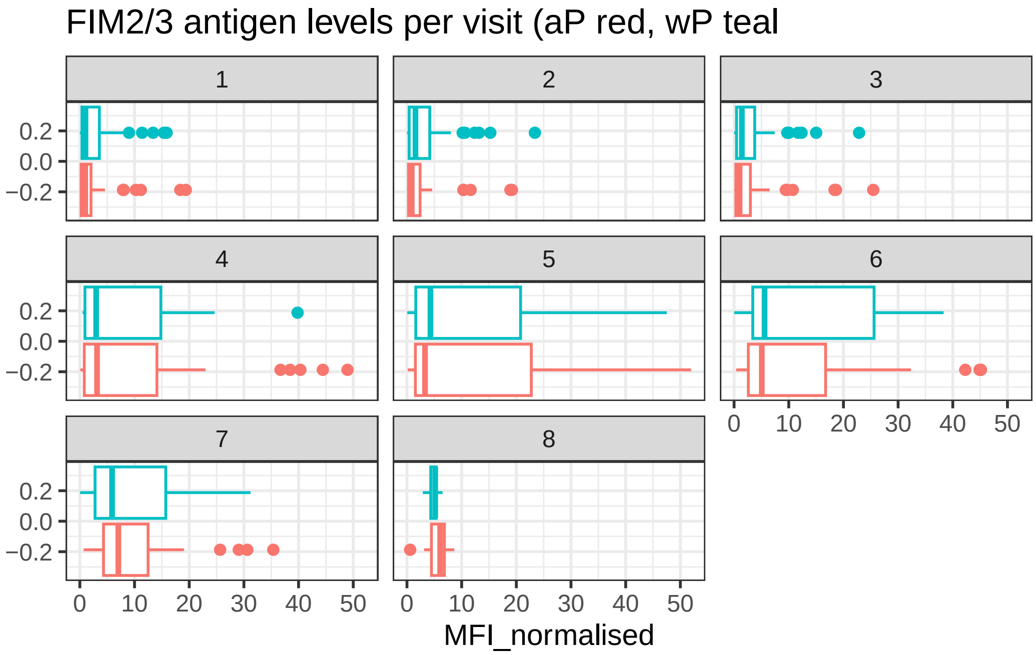


doing the same, but only looking at 8 visits

```
filter(igg_8, antigen=="OVA") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  labs(title = "OVA antigen levels per visit (aP red, wP teal")
```

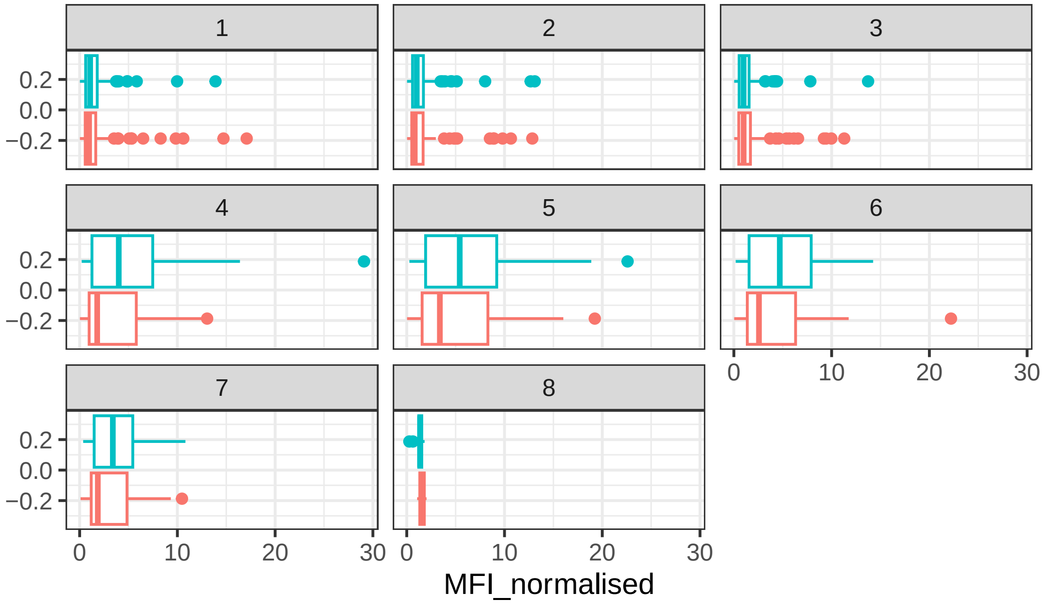


```
filter(igg_8, antigen=="FIM2/3") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  labs(title = "FIM2/3 antigen levels per visit (aP red, wP teal")
```



```
filter(igg_8, antigen=="PT") %>%
  ggplot() +
  aes(MFI_normalised, col=infancy_vac) +
  geom_boxplot(show.legend = FALSE) +
  facet_wrap(vars(visit)) +
  theme_bw() +
  labs(title = "PT antigen levels per visit (aP red, wP teal")
```

PT antigen levels per visit (aP red, wP teal



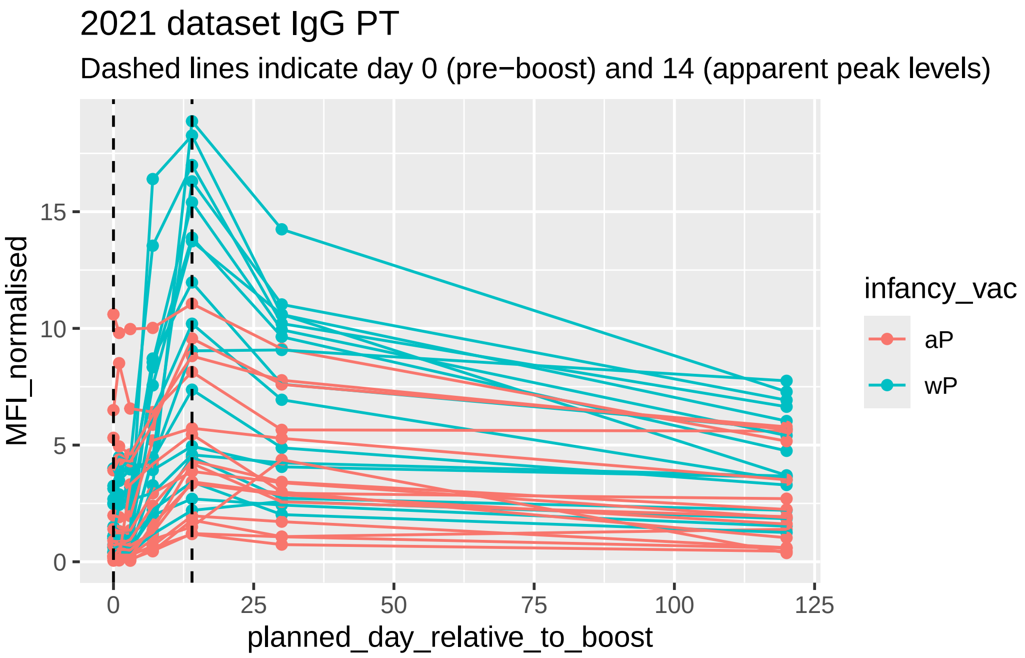
Q16. What do you notice about these two antigens time courses and the PT data in particular?

OVA levels do not change much from visit to visit, but the PT and FIM2/3 levels increase at first, peak around visit 5/6, and then decrease after that. The PT and FIM2/3 levels are much higher than the OVA levels as well.

Q17. Do you see any clear difference in aP vs. wP responses?

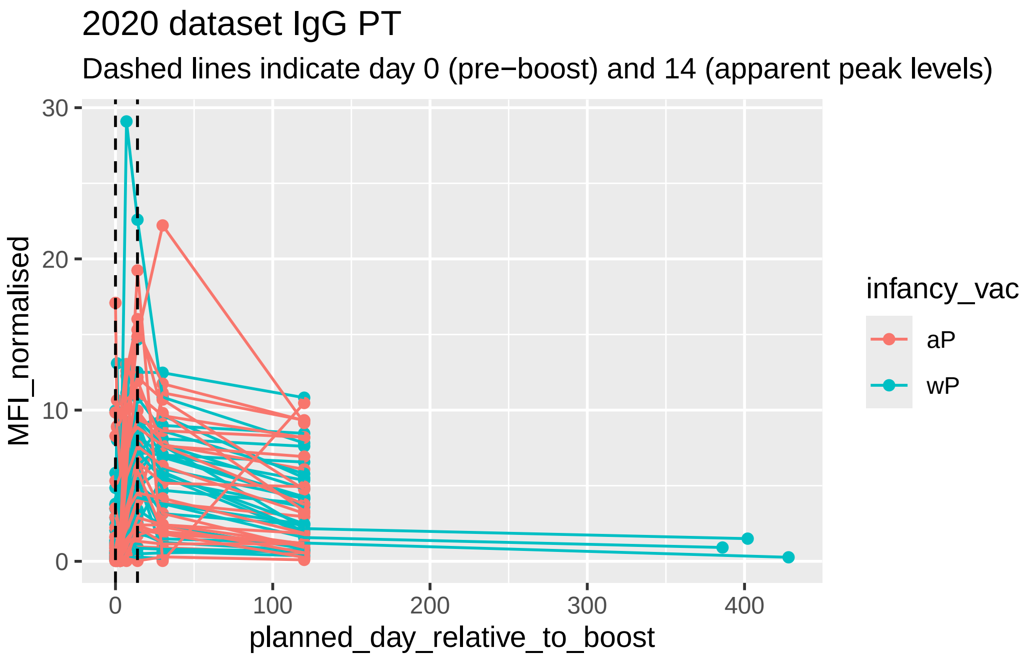
No, the aP and wP have similar responses in the levels of OVA, PT, and FIM2/3 over time.

```
abdata.21 <- abdata %>% filter(dataset == "2021_dataset")

abdata.21 %>%
  filter(isotype == "IgG",  antigen == "PT") %>%
  ggplot() +
    aes(x=planned_day_relative_to_boost,
        y=MFI_normalised,
        col=infancy_vac,
        group=subject_id) +
    geom_point() +
    geom_line() +
    geom_vline(xintercept=0, linetype="dashed") +
    geom_vline(xintercept=14, linetype="dashed") +
  labs(title="2021 dataset IgG PT",
       subtitle = "Dashed lines indicate day 0 (pre-boost) and 14 (apparent peak levels)")
```



Q18. Does this trend look similar for the 2020 dataset?

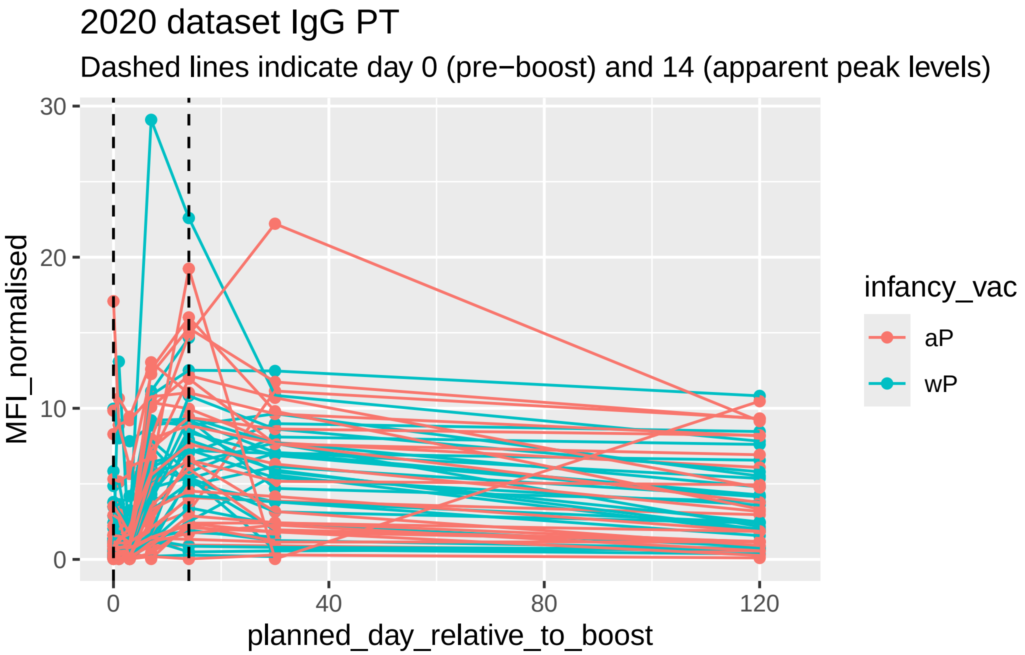
```
abdata.20 <- abdata %>% filter(dataset == "2020_dataset")

abdata.20 %>%
  filter(isotype == "IgG",  antigen == "PT") %>%
  ggplot() +
    aes(x=planned_day_relative_to_boost,
        y=MFI_normalised,
        col=infancy_vac,
        group=subject_id) +
    geom_point() +
    geom_line() +
    geom_vline(xintercept=0, linetype="dashed") +
    geom_vline(xintercept=14, linetype="dashed") +
  labs(title="2020 dataset IgG PT",
       subtitle = "Dashed lines indicate day 0 (pre-boost) and 14 (apparent peak levels)")
```



```
abdata.20 <- abdata %>% filter(dataset == "2020_dataset")

abdata.20 %>%
  filter(isotype == "IgG",  antigen == "PT") %>%
  ggplot() +
    aes(x=planned_day_relative_to_boost,
        y=MFI_normalised,
        col=infancy_vac,
        group=subject_id) +
    geom_point() +
    scale_x_continuous(limits = c(NA, 125)) +
    geom_line() +
    geom_vline(xintercept=0, linetype="dashed") +
    geom_vline(xintercept=14, linetype="dashed") +
  labs(title="2020 dataset IgG PT",
       subtitle = "Dashed lines indicate day 0 (pre-boost) and 14 (apparent peak levels)")
```

```
Warning: Removed 3 rows containing missing values or values outside the scale range
(`geom_point()`).
```

```
Warning: Removed 3 rows containing missing values or values outside the scale range
(`geom_line()`).
```



The trend is mostly similar between 2021 and 2020 where the levels peak and then go back down. 2020 does have a few samples where the planned day relative to boost goes out to ~400. It does seem like in the 2020 dataset, the peak is a little before 14 days, but it is still overall pretty similar to 2021.

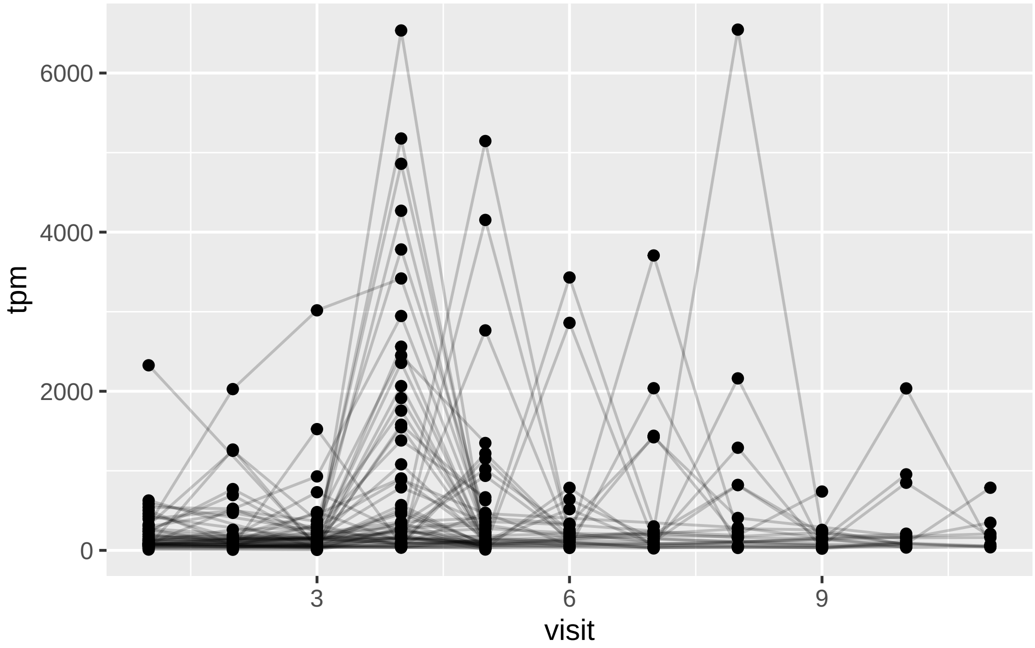
```
url <- "https://www.cmi-pb.org/api/v2/rnaseq?versioned_ensembl_gene_id=eq.ENSG00000211896.7"

rna <- read_json(url, simplifyVector = TRUE)
```

```
#meta <- inner_join(specimen, subject)
ssrna <- inner_join(rna, meta)
```

```
Joining with `by = join_by(specimen_id)`
```

Q19. Make a plot of the time course of gene expression for IGHG1 gene (i.e. a plot of visit vs. tpm)

```
ggplot(ssrna) +
  aes(x=visit, y=tpm, group=subject_id) +
  geom_point() +
  geom_line(alpha=0.2)
```



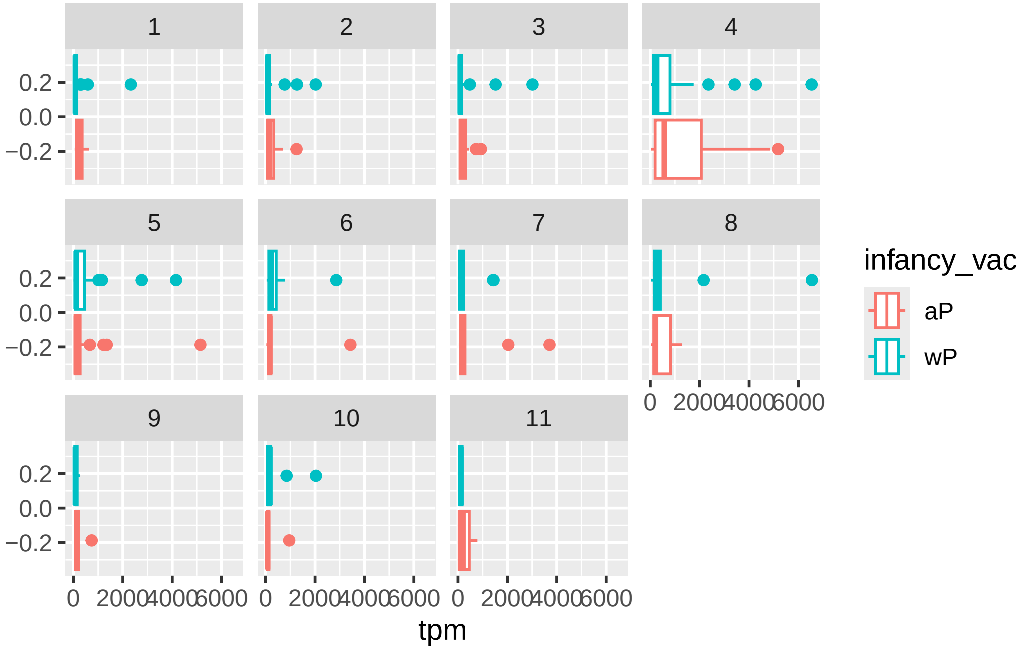
Q20.: What do you notice about the expression of this gene (i.e. when is it at it's maximum level)?

The gene is at its maximum expression level around visit 4 for the most part.

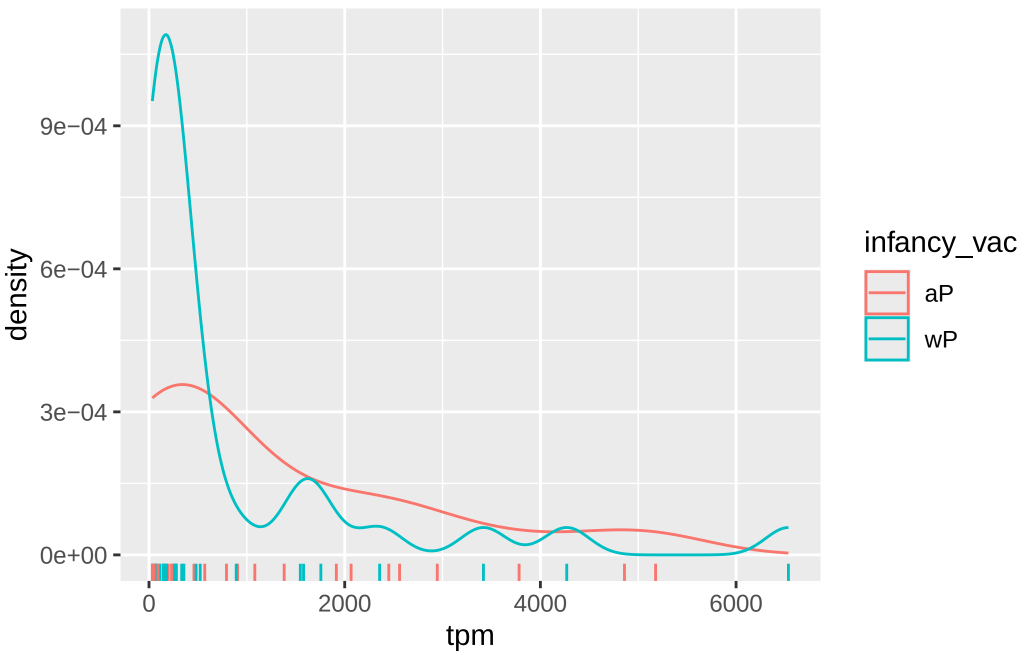
Q21. Does this pattern in time match the trend of antibody titer data? If not, why not?

The antibody titer data peaks around visit 5/6, whereas the gene expression peaks around visit 4. This makes sense because the cells would first express the gene more to make more antibodies that would then hang around for longer than the gene is overexpressed.

```
ggplot(ssrna) +
  aes(tpm, col=infancy_vac) +
  geom_boxplot() +
  facet_wrap(vars(visit))
```



```
ssrna %>%
  filter(visit==4) %>%
  ggplot() +
    aes(tpm, col=infancy_vac) + geom_density() +
    geom_rug()
```


density
9e−04
6e−04
3e−04
0e+00
0
2000
4000
6000
tpm
infancy_vac
aP
wP